

NO-A143 724

THE USE OF GENETIC MECHANISMS AND BEHAVIORAL
CHARACTERISTICS TO CONTROL N. (U) VIRGINIA POLYTECHNIC
INST AND STATE UNIV BLACKSBURG DEPT OF E.
N H ROSS ET AL. AUG 84 N00014-77-C-0246

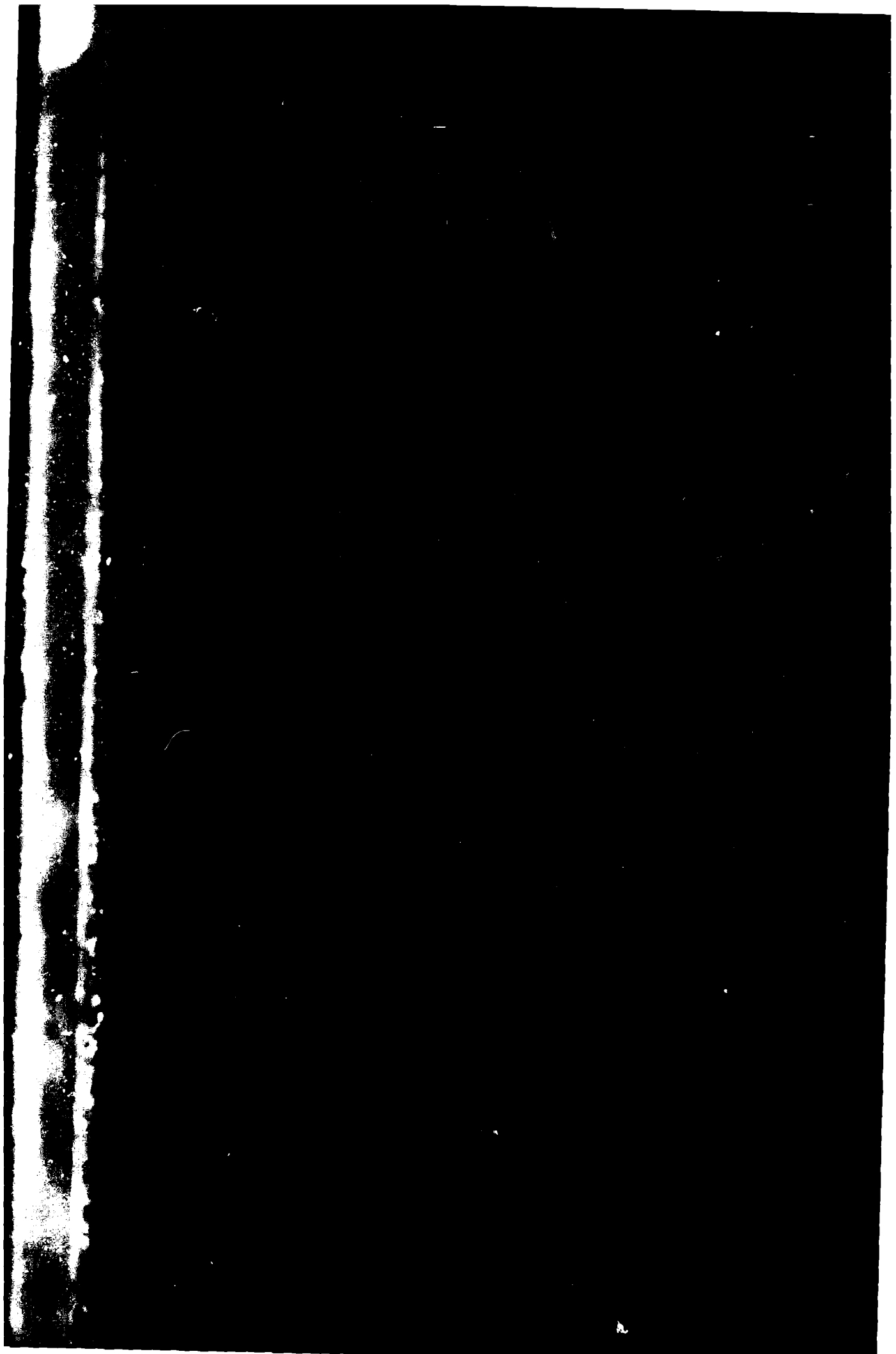
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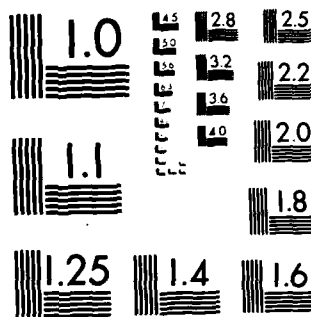
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MICROCOPY RESOLUTION TEST CHART
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by

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August 1984

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This research was supported in part by the Office of Naval Research, Microbiology Program, Naval Biology Project under Contract N00014-77C-9246.

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The research conducted during this Contract year is a continuation of studies on the behavior of German cockroach (*Blattella germanica* (L.)) populations. Natural populations contain nymphs in 6-7 stadia, adult males, and adult females that are generally in different stages of their reproductive cycles. Our investigations have revealed differences in the production and response to naturally-occurring (pheromonal) and extrinsic (insecticidal) chemical stimuli with nymphal stage, adult sex, and female reproductive state. Population behavior of this species is much more complex than realized heretofore.

Currently we are conducting laboratory experiments on the response of six age/sex classes to chemical cues produced by adult females. Other experiments are testing for possible differences in dispersal and other behaviors with age/sex class and strain (susceptible vs propoxur resistant) following exposure to propoxur vapors. Data from prior Navy-supported research are either being prepared or have been submitted for publication.

Insecticide-Induced Dispersal

Insecticides have repellent effects. This has led to a widely held but poorly substantiated belief that cockroaches have developed "behavioral" resistance. That is, it is presumed that selection pressure in the field has altered behavior to give the insects a better chance of survival, possibly by dispersing faster or farther than those of the original strains. In summarizing experiments conducted on an inactive ship in 1982 and 1983, we find no evidence of a difference in dispersal between members of a laboratory susceptible and a propoxur resistant field strain following treatment with propoxur. Higher survival of the "field" strain was clearly due to physiological resistance. The apparent absence of behavioral changes does not prove that behavioral resistance hasn't developed in other "field" strains, but it does show that this possibility needs to be examined critically. Laboratory experiments by the student supported by this Contract (B. L. Bret) show no significant difference between dispersal of mixed age groups of the resistant and susceptible strains (Fig 1). However, when the results are separated according to specific age/sex classes, it appears that adults of the susceptible strain disperse somewhat more than those of the resistant strain - the opposite of the behavior that would be expected if adults of the resistant strain are better able to escape the insecticide. An interesting difference is also beginning to emerge in observations on the number of antennal and tarsal cleanings in adult males of the two strains, but these experiments are not yet completed.

Population Behavior:

Our first field experiment on wild-type population behavior was conducted on an inactive ship in 1981¹. Differences were observed in the behavior of nymphal age classes and also between

adult females with and without egg cases. For example, middle-late stage nymphs were more widely distributed and apparently moved more frequently from one location to another than other age/sex classes. Nearly all females with egg cases were in aggregations that were close to water and food. Laboratory experiments provided supporting evidence of behavioral differences between the members of mixed age groups^{2 3}. The results correlated well with "field" observations. In addition, group behavior was influenced by the density and reproductive state of the adult females. The importance of the role played by the adult female in population behavior has been called to the attention of pest control operators⁴.

In the course of the above research, it became increasingly clear that population behavior in *B. germanica* is explicable only in terms of behavioral patterns of its members. The most likely determinants of the observed behaviors are the aggregation pheromone and a dispersant. Bioassay experiments are being used to test for varying responses to these chemical cues among the following age/sex classes: small nymphs (1st-2nd stadia); mid-stage nymphs (3rd-4th stadia); large nymphs (5th-6th stadia); adult males; and females with (gravid) and females without egg cases (non-gravid). The experiments utilized filter papers that had been conditioned by adult females. Both gravid and non-gravid females were used in order to investigate possible differences in the production of chemical stimuli with reproductive state.

Aggregation pheromone. Differences in the rate, strength, and arrestant effects of the response to the aggregation pheromone were evaluated by comparing the proportions of each age/sex class that were on papers conditioned by 10 adult females (one paper conditioned by non-gravid and another by gravid females). A third non-conditioned paper was also used and any cockroaches not on conditioned papers were recorded as being either on the control paper or the bottom of the jar in which the experiment was conducted. All test classes formed a stabilized distribution within 1 1/2 hrs., but adult males and mid-stage nymphs showed little change after the first 1/2 hr. The strongest response was that of small nymphs, with a mean of 98% that settled on the conditioned papers. Large nymphs, adult males, and gravid females also responded strongly (means ranging from 92.0% to 95.5%). No significant difference occurred among the latter. The response of non-gravid females was weaker (mean 91.0%). Least response was that of mid-sized nymphs (mean 85.1%). Table 1 shows this behavior analyzed from the standpoint of relative proportions of each class that were not attracted to the conditioned papers. After distribution was stabilized (1 1/2 hrs.), the number of small nymphs on conditioned papers was more constant than those of mid-stage nymphs. This evidence of lesser movement among small nymphs is probably correlated with their higher attraction to aggregation pheromone, since the pheromone is known to act as an arrestant as well as an attractant.

Analysis of the relative attraction to the papers exposed to gravid vs non-gravid females was complicated by a tendency of some age classes to form a single major aggregation on either one or the other of the conditioned papers (Table 2). This behavior was particularly marked among small nymphs where 33% of the observations showed all were on the same paper. Adults showed a similar tendency. It is as if where one insect settled, others tended to follow. Mid-stage nymphs did not show this behavior and late instars showed only a slight tendency in this direction. Possibly there is some type of intra-attraction among particular age-sex classes or, alternatively, a difference in their ability to sense trails. If the latter explanation is correct, the absence of the behavior among mid-stage nymphs is probably related to their weak response to the pheromone.

Table 3 shows comparisons of responses to papers conditioned by gravid vs non-gravid females. Only two of the test classes, mid-stage nymphs and non-gravid females, showed no difference in their response. The other classes responded more strongly to the paper conditioned by non-gravid females. Aggregation pheromone is excreted in the feces, and there was more fecal material on the papers conditioned by non-gravid females. It can be reasonably concluded that the greater attraction to papers conditioned by these females was due to a higher concentration of aggregation pheromone. Production of the pheromone apparently varies with female reproductive state. The absence of a higher attraction to the paper conditioned by non-gravid females among middle stage nymphs and non-gravid females suggests that, in addition to a weaker response to the pheromone, the upper threshold of their response is lower than that of other age/sex classes. Comparisons between the classes show a very strong response of males to the paper conditioned by non-gravid females. Since female sex pheromone is produced prior to egg case formation, it is possible the attraction of males was due in part to female sex pheromone.

Behavior of mid-stage nymphs seen in "field" and laboratory experiments, i.e., their relatively high dispersal and wider distribution within a harborage, can now be attributed to a weaker response to aggregation pheromone, combined with a relatively low upper threshold of the response. Likewise, the strong within-harborage aggregation and low dispersal of small nymphs is explained by their very strong response to the pheromone.

We suggest that the observed behavioral patterns reflect adaptive strategies that have been developed in the course of a long evolutionary history; that is, they are characteristic of the species. The results on mid-stage nymphs are reminiscent of advantageous adaptive strategies of flying insects where dispersal occurs "while flight system is maximized and that of the reproductive system minimized"⁵. Mid-stage nymphs have the longest period for dispersal after very early stages when chances

of survival would be poor and before females mature and form egg cases. The strong response to aggregation pheromone might act to keep gravid females and their newly hatched nymphs in locations favorable to survival, i.e., near water and food.

Pheromone-like dispersant. - The dispersant is produced under conditions of stress⁶. In our experiments, filter papers were conditioned by crowding 30 gravid or non-gravid adult females into small vials that contained the paper. Fig. 2 shows the results of 10 replicates of 10 early instars/relicate. These results are representative of the experiments with mid-stage and large nymphs, and also with gravid females. Two additional replicates (total of 12) will be completed for each test class before the end of the contract year. The insects were repelled from both papers, but the greater repellency was associated with that conditioned by non-gravid females. Apparently non-gravid females produce more of the dispersant than gravid females. It masked the effect of aggregation pheromone, presuming the latter is indeed produced under these conditions. The response of adult males differed from the above age/sex classes in that the relative repellency of the two conditioned papers was reversed. Possibly female sex pheromone on the paper conditioned by non-gravid females countered that of the dispersant, as well as adding to the attraction of the paper in the aggregation experiments. Non-gravid females were equally repelled from the two conditioned papers.

Since the dispersant is produced under stress, it is hardly surprising that the response to this signal of unfavorable or dangerous situations does not differ greatly with age/sex class.

In the original report of the German cockroach dispersant⁶, it was obtained by stressing "adults". We are examining this situation further in order to determine whether it is produced by adult males as well as females and whether nymphs are capable of giving this signal. Filter papers are being conditioned by crowding 40 adult males and 50 large nymphs into small vials for 1 hr, instead of 30 individuals as used in the experiments with females. Preliminary data suggest that members of the test group (adult males) are not repelled by either of the conditioned papers. It will indeed be interesting if the adult females are the only members of a population to give this signal of stress. Preliminary analytical work, using spectrophotometry, to compare female-produced saliva (containing dispersant) to that produced by males (?apparently lacking the dispersant) is in progress.

Citations

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2. Influence of adult females on within-shelter distribution patterns of Blattella germanica (Dictyoptera: Blattellidae). B. L. Bret, M. H. Ross, and G. I. Holtzman. Ann. Entomol. Soc. Amer 76:847-852. 1983.*
3. A laboratory study of German cockroach dispersal (Dictyoptera; Blattellidae). B. L. Bret and M. H. Ross. Subm. Proc. Entomol. Soc. Wash.*
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5. Insect Behavior. R. W. Matthews and J. R. Matthews. John Wiley & Sons, N. Y. 1978. 507 pp.
6. Secretion of dispersion-inducing substance by the German cockroach, Blattella germanica (L.) (Orthoptera: Blattellidae). C. Suto and N. Kumada. Appl. Ent. Zool. 16:113-120. 1981.

*Research conducted under this Contract. Additional reports include:

- Cochran, D. G. 1983. Food and water consumption during the reproductive cycle of female German cockroaches. Ent. exp. appl. 34:51-57. 1983.
- Durban, E. J. and D. G. Cochran. 1984. Food and water deprivation effects on reproduction in female Blattella germanica (L.) Ent. exp. appl. (In press).

Table 1. Comparative strength of responses to conditioned papers in the aggregation experiments.

Age/sex class ¹	Not on condit. papers (mean %) ²	Response
Mid-stage nymphs	12.89 a	weakest
Non-gravid females	5.59 b	
Adult males	2.74 c	
Gravid females	2.20 c	
Large nymphs	1.84 c	
Small nymphs	1.68 c	strongest

¹ Mid-stage nymphs - 3rd-4th stadia; non-gravid females - females without egg cases; gravid females - females with egg cases; large nymphs - 5th-6th stadia; small nymphs - 1st-2nd stadia.

² Significant differences at $P > 0.05$ indicated by "a", "b", or "c".

Table 2. Tendency to form a single major aggregation on one of the conditioned papers.

Age/sex class	No.	Observations ¹		
		All on 1 paper	Preponderance on 1 paper ²	From 75-100% on 1 paper
Small nymphs	60	33.3%	50.0	83.3%
Mid-stage nymphs	80	0	52.5	52.5%
Large nymphs	120	8.3%	57.5	65.8%
Adult males	240	27.5%	40.8	68.3%
Non-gravid females	240	24.6%	49.6	74.2%
Gravid females	240	30.8%	40.4	71.2%

¹ 10 observations/replicate (30 min intervals from 1.5 to 6 hrs).

Includes only cockroaches that were on the conditioned papers.

² At least 75%/one paper but excluding the "all as none response" (col. 3).

Table 3. Comparison of responses to papers conditioned by gravid and non-gravid females (aggregation exp'ts).

Age/sex class	Response (%)	
	Paper condit. by non-gravid ♀	Paper condit. by gravid ♀
Small nymphs ²	45.3 b ³	28.1
Mid-stage nymphs	50.0 b,c	42.1
Large nymphs ²	56.2 b	33.1
Adult males ²	66.6 a	18.9
Non-gravid females	37.6 d	42.1
Gravid females ²	45.1 c	33.3

¹ Avg. % on each paper at each time interval divided by the no. of time intervals (10).

² Significantly higher proportions on papers conditioned by non-gravid females. ($P > 0.05$).

³ Letters indicate significant differences between age/sex classes ($P > 0.05$).

Fig. 1. Comparison between the proportions of two strains of cockroaches that failed to disperse following exposure to propoxur vapors.

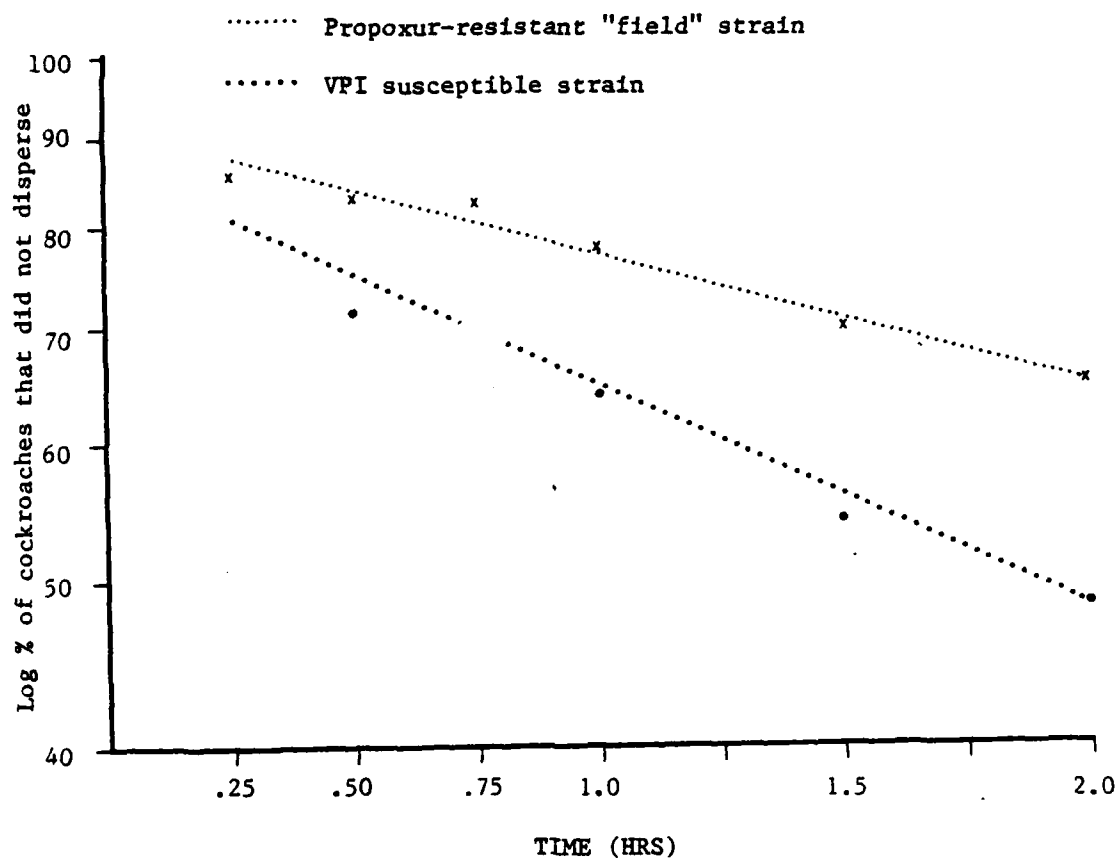
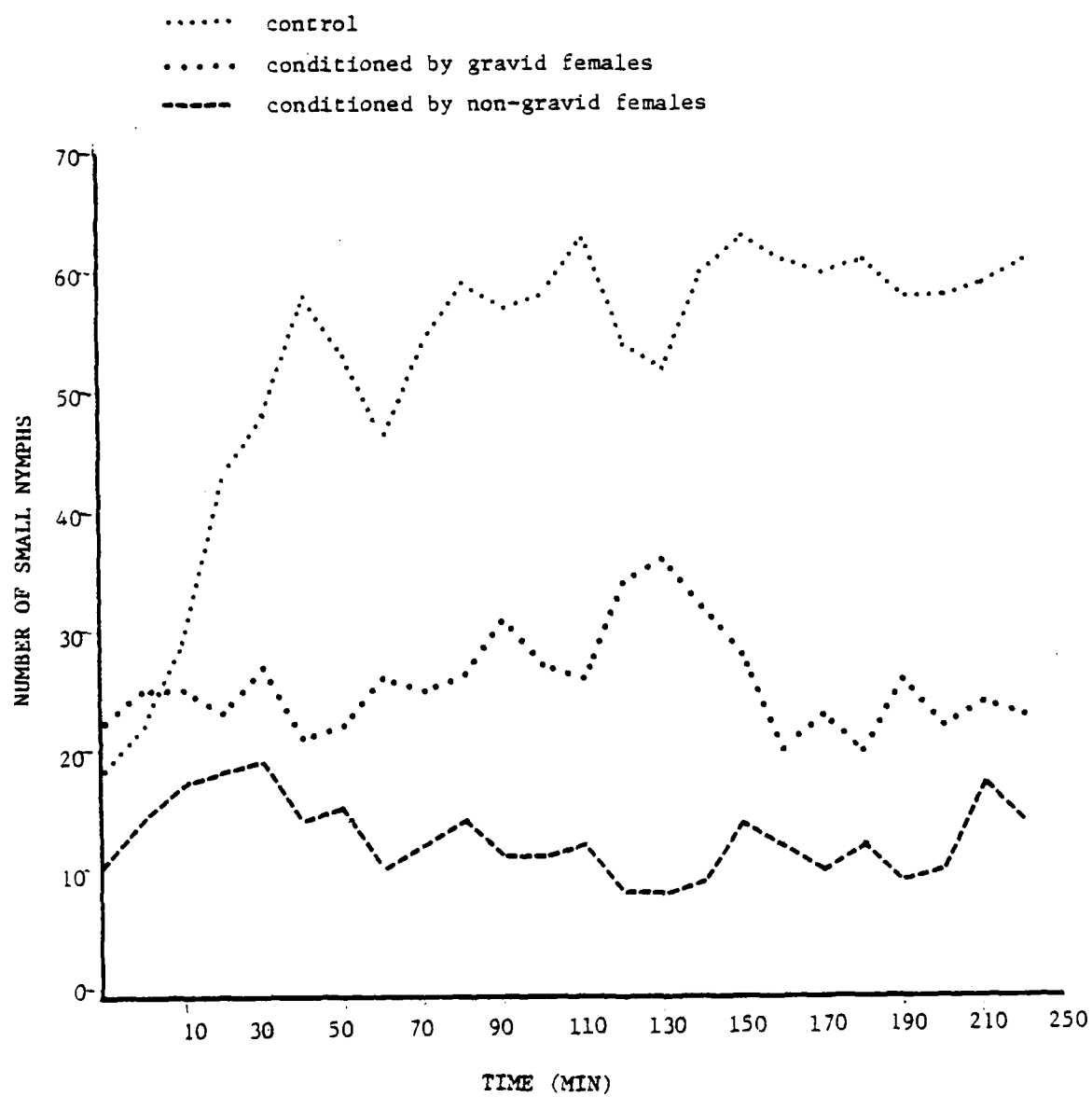


Fig. 2. Response of small nymphs to papers conditioned by crowding adult females.



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